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The goal of this research is to develop thermodynamically correct bioavailability estimations using chromatographic stationary phases as a model of the "interphase" system. It has been previously established that octanol-water partition coefficients are not thermodynamically relevant for the modeling of bioaccumulation processes (Opperhuizen et al., *Environ. Sci Technol.* **1988**, 22, 286). They investigated the thermodynamic properties of the partitioning of chlorobenzenes between fish lipids and water and showed that bioconcentration is accompanied by *positive* enthalpy and entropy changes. In contrast, the partitioning of these compounds between octanol and water is accompanied by *negative* enthalpy and by small negative or positive entropy changes. They conclude that the differences in the thermodynamic properties of these processes arise from the different structures of fish lipids and octanol, and that only under very specific conditions and only for structurally similar compounds can a relationship between octanol-water partitioning and bioaccumulation be expected. We have spent the past eight years investigating the molecular mechanism of retention of reversed phase liquid chromatology (RPLC) and have shown that at sufficiently high bonded chain density, partitioning of solutes to reversed phase chromatographic stationary phases match the partitioning thermodynamics between fish lipids and water measured by Opperhuizen.

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Year End Report
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"Thermodynamically Correct Bioavailability Estimations"

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The goal of this research is to develop thermodynamically correct bioavailability estimations using chromatographic stationary phases as a model of the "interphase" system. It has been previously established that octanol-water partition coefficients are not thermodynamically relevant for the modeling of bioaccumulation processes (Opperhuizen et al., *Environ. Sci. Technol.* **1988, 22**, 286). They investigated the thermodynamic properties of the partitioning of chlorobenzenes between fish lipids and water, and showed that bioconcentration is accompanied by *positive* enthalpy and entropy changes. In contrast, the partitioning of these compounds between octanol and water is accompanied by *negative* enthalpy and by small negative or positive entropy changes. They conclude that the differences in the thermodynamic properties of these processes arise from the different structures of fish lipids and octanol, and that only under very specific conditions and only for structurally similar compounds can a relationship between octanol-water partitioning and bioaccumulation be expected.

We have spent the past eight years investigating the molecular mechanism of retention of reversed phase liquid chromatography (RPLC), and have shown that at sufficiently high bonded chain density, partitioning of solutes to reversed phase chromatographic stationary phases match the partitioning thermodynamics between fish lipids and water measured by Opperhuizen.

During this second year of our AFOSR support, we have made significant progress toward our goals of using our well characterized stationary phases for modeling bioaccumulation processes. Specifically, we have made advances in three areas.

First, we have spent considerable time further investigating the thermodynamics of partitoning processes of small molecules between bulk solutions and our chromatographic stationary phases. During this past year we had two major manuscripts published by ANALYTICAL CHEMISTRY (numbers 1 & 2, below) detailing this work and acknowledging AFOSR support. The first of these showed both chromatographic temperature studies and differential scanning calorimetry experiments investigating the role of alkyl chain bonding density on the retention mechanism of RPLC. Phase transitions of these stationary phases were observed at bonding densities greater than 2.84 μmole/m^2. Thermodynamic constants for the transfer of a solute from the mobile phase to the stationary phase ($\Delta H°$ and $\Delta S°$) were calculated for low bonding density columns, and comparison of these values to previously reported values for the partitioning of a nonpolar solute from the bulk organic liquid to water indicated that the chromatographic retention process is not well-modeled by bulk phase oil-water partitioning processes. In addition, this data showed that the entropic contribution to retention becomes more significant with respect to the enthalpic contribution as the stationary phase bonding density is increased, providing additional support that partitioning, rather than adsorption, is the relevant model of retention. The second paper examined temperature effects over a wide range, with emphasis on the role of the mobile phase. Van't Hoff plot shapes were used to assess the retention mechanism, and the data showed evidence of the hydrophobic effect when water-rich and/or hydrogen-bonded mobile phases such as methanol/water were used. However, different

van't Hoff plot shape was observed with acetonitrile/water mobile phases, indicating a change in the retention mechanism. These data showed that the hydrophobic effect, which had been previously proposed as the driving force for retention, is not a satisfactory explanation for the retention process in all RPLC systems.

Second, we have finished a very thorough investigation of the best chromatographic measure to use for correlation with biological partitioning values. This work has just been published in the JOURNAL OF CHROMATOGRAPHY (number 4, below). Use of chromatographic retention requires that a standard set of mobile phase conditions be chosen. The choice of 100% water has theoretical advantages, as an aqueous phase-membrane phase is the most common system being modeled. However, experimental measurement of k' (retention) values with this mobile phase is difficult or impossible for most real solutes. Various retention extrapolation methods to 100% water have been proposed, but when compared, often yield different values for the same solute. Most of the extrapolation methods are based on the retention as a function of the mobile phase only. However, as the retention is controlled by solute partitioning between the mobile phase and stationary phase, stationary phase effects cannot be ignored. We have compared log k'_w, the retention value in 100% water, using different extrapolation methods and compared these with experimentally measured values. Prediction of log k'_w is attempted from the retention as a function of both the mobile phase and stationary phase. Solvatochromic analysis is used to deconvolute stationary and mobile phase effects, and we have concluded that log k'_w values extrapolated from a solvatochromic measure we proposed several years ago are the most meaningful representation of retention for quantitative structure-retention relationships.

Thirdly, we have spent considerable time investigating the use of mobile phase additives for the purpose of increasing the stationary phase chain density *in situ*. This work may become especially important, as commercially available stationary phases are of sufficiently low chain density that they do not show the appropriate thermodynamic partitioning values. We have just published the first part of this work in the JOURNAL OF CHROMATOGRAPHY (number 5, below). Here we investigated the effect of stationary phase solvation on reversed phase chromatographic shape selectivity, using n-hexanol as an additive to methanol-water mobile phases. A wide range of mobile phase compositions was evaluated to normalize for solvent strength selectivity differences. Monomeric C-18 stationary phases of both high and low bonding density were synthesized and used to correlate selectivity changes caused by stationary phase ordering with those seen by the addition of n-hexanol. The temperature dependence of retention and selectivity was also investigated using van't Hoff plots, which provided insight into the nature of selectivity behavior for estrogens and polyaromatic hydrocarbons. The results showed that using n-hexanol as a mobile phase additive did not provide higher shape selectivity, suggesting that changes in the solvation of the stationary phase did not impart significant change in the level of surface ordering or morphology. However, n-hexanol did impart solvent selectivity changes in the separation of estrogen diastereomers which could prove useful in future methods development schemes.

We have also just concluded a thorough investigation of the use of cholesterol as a mobile phase additive. This work is now being written for submission for publication. In brief, we have found that low chain density stationary phases "take-up" a significant amount of cholesterol, and significantly change the chromatographic selectivity, which is a function of the thermodynamics. This gives us great hope that the use of this additive will make commercially available reversed phase columns act as our "high-density" columns, and will show the correct thermodynamic properties for modelling of biological partitioning processes.

In addition, we have two manuscripts in press for a special issue of the JOURNAL OF CHROMATOGRAPHY (numbers 6 & 7, below), which will be devoted to retention mechanisms of reversed phase liquid chromatography. These manuscripts are critical reviews of

(1) phase transition phenomena, including molecular modeling approaches to understanding the important remaining issues; and (2) hydrophobicity estimations by reversed phase LC. This area is the central theme of research supported by this grant, and this review is a thorough review of work accomplished since 1986. We have also published a major review of all fundamental LC work performed in the past two years (number 3, below). This was published in the Fundamental Review issue of ANALYTICAL CHEMISTRY, and acknowledges AFOSR support. We are the senior invited author for this review; the Fundamental Review issue is published by ANALYTICAL CHEMISTRY in even-numbered years, and contains only invited reviews of the important analytical techniques.

During the third year of our grant, we propose to continue the cholesterol studies, and we also have a significant effort underway to correlate biological partitioning processes with chromatographic retention on a variety of stationary phases. This work should result in several significant publications during the coming year.

Publications Acknowledging AFOSR Support:

1. "Temperature Dependence of Retention in Reversed Phase Liquid Chromatography: Mobile Phase Effects", Lynn A. Cole and John G. Dorsey, **ANAL. CHEM.,** 1992, 64, 1317-1323.

2. "Temperature Dependence of Retention in Reversed Phase Liquid Chromatography: Stationary Phase Effects", Lynn A. Cole, John G. Dorsey and Ken A. Dill, **ANAL. CHEM.,** 1992, 64, 1324-1327.

3. "Liquid Chromatography: Theory and Methodology", John G. Dorsey, Joe P. Foley, William T. Cooper, Robert A. Barford and Howard G. Barth, **ANAL. CHEM.** 1992, 64, 353R-389R.

4. "Accurate Determination of log k'_w in Reversed Phase Liquid Chromatography: Implications for Quantitative Structure Retention Relationships", Mei-Ming Hsieh and John G. Dorsey, **J. CHROMATOGR.,** 1993, 631, 63-78.

5. "The Effect of Stationary Phase Solvation on Shape Selectivity in Reversed Phase Liquid Chromatography", Steven R. Cole and John G. Dorsey, **J. CHROMATOGR.**, 1993, 635, 177-186.

6. "Phase Transitions of Reversed Phase Stationary Phases: Cause and Effects in the Mechanism of Retention", John F. Wheeler, Thomas L. Beck, S. J. Klatte, Lynn A. Cole and John G. Dorsey, **J. CHROMATOGR.,** in press, 1993.

7. "Hydrophobicity Estimations by Reversed Phase Liquid Chromatography: Implications for Biological Partitioning Processes", John G. Dorsey and Morteza G. Khaledi, **J. CHROMATOGR.,** in press, 1993